UDC 593.176

MORPHOMETRIC VARIATIONS OF *DISCOPHRYA ELONGATA* (CILIOPHORA, SUCTOREA) ATTACHED TO TWO DIFFERENT SPECIES OF AQUATIC TRUE BUGS (HEMIPTERA, PROSORRHYNCHA, NEPOMORPHA)

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Received 27 January 2011
Accepted 30 March 2011

Morphometric Variations of *Discophrya elongata* (Ciliophora, Suctorea) Attached to Two Different Species of Aquatic True Bugs (Hemiptera, Prosorrhyncha, Nepomorpha). Marico-Pérez R., Mayén-Estrada R., Macip-Ríos R., Dovgal I. V. — Morphometric variation in *Discophrya elongata* living as epibionts of two species of aquatic true bugs, *Corisella edulis* and *Notonecta unifasciata*, collected from the same pond in Mexico are discussed. Factors that may be responsible for observed variability, especially hydrodynamic loads and long-term modifications, also are identified and discussed.

Key words: *Discophrya elongata*, suctorian, variability, host, hydrodynamic loads, long-term modifications.

Изменчивость *Discophrya elongata* (Ciliophora, Suctorea) при поселении на двух разных видах водных клопов (Hemiptera, Prosorrhyncha, Nepomorpha). Марино-Перес Р., Майен-Эстрада Р., Масип-Риос Р., Довгаль И. В. — В статье обсуждается изменчивость размерных характеристик полиморфного вида сукторий *Discophrya elongata* при поселении на двух видах водных клопов *Corisella edulis* и *Notonecta unifasciata* из одного местообитания — пруда в Мексике. Указаны факторы, с которыми могут быть связаны наблюдаемые различия в размерах вида, в частности, гидродинамические нагрузки и длительные модификации.

Ключевые слова: *Discophrya elongata*, суктория, изменчивость, хозяин, гидродинамические нагрузки, длительные модификации.

Introduction

Intraspecific variation is poorly understood in ciliates, especially in commensals or parasitic species. Variation in size and shape of the cell body, length of the stalk, and numbers of contractile vacuoles according to the nature of the substrate, host species, and localization on the host body have been reported in suctorian ciliates such as *Discophrya lichtensteinii* (Claparède et Lachmann, 1859) (Matthes, 1954a; Dovgal, Kochin, 1997; Dovgal, 2008), *Periacineta buckei* (Kent, 1882) (Matthes, 1954b); *Setodiscophrya erlangensis* (Matthes, 1954) (Matthes and Plachter, 1975) and *Dendrosoma radians* Ehrenberg, 1838 (Dovgal, 2002).

Discophrya elongata (Claparède et Lachmann, 1859) is among the most polymorphic suctorian species. Claparède, Lachmann (1859) observed it (as *Podophrya elongata*) on shells of the mollusk *Viviparus viviparus* (Linnaeus, 1758) (as *Paludina vivipara*). The species was described as a suctorian with a cell body nearly five or six times longer than its width, bearing tentacles at the apex of the body, and with two opposing bundles of tentacles at the body's equator. The macronucleus was described as ribbon-like, and the cells had numerous contractile vacuoles.

The high degree of variability among individual cells of this species was pointed out by Claparède, Lachmann (1859). They mentioned that the stalk of *D. elongata* may be longitudinally or transversely striated, and although

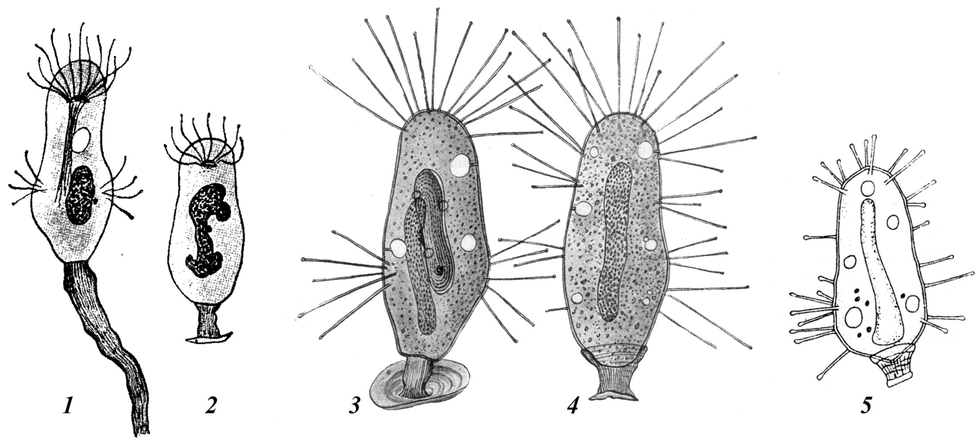


Fig. 1. *Discophrya elongata*: *1–2* — after Collin, 1911; *3–4* — after Collin, 1912; *5* — after Rieder, 1936.
Рис. 1. *Discophrya elongata*: *1–2* — по: Collin, 1911; *3–4* — по: Collin, 1912; *5* — по: Rieder, 1936.

the stalk as a rule was not longer than one-third the length of the body, individuals with stalks twice as long as the body also were observed.

Collin (1911) addressed variability of several suctorian species, including *D. elongata.* He found the species on shells of *Lymnaea auricularia* (Linnaeus, 1758) from a pond in the Munich Botanical Garden (Germany) and noted significant variability in the size of the body, length of the stalk, and shape of the macronucleus (fig. 1, *1–2*).

The variability of *D. elongata* is reflected in its nomenclatural history. In his monograph on suctorians, Collin (1912) described a new variety of *D. elongata* from shells of the mollusk *Contectiana contecta* (Millet, 1813) (as *Vivipara contecta*), from a pond in the Montpellier Botanical Garden (France). *Discophrya elongata* var. *scyphostyla* Collin, 1912 differs from the typical form in possessing a short (20–25 μm), robust stalk that occasionally shows a cup-like expansion at the point where it attaches to the aboral end of the cell body (fig. 1, *3–4*).

Penard (1920) raised Collin's variety to specific level as *D. scyphostyla* Collin, 1912. Subsequently, Rieder (1936) found the same morphotype on materials collected in Etang du Jura near Freiburg, Switzerland and described as a new species, *D. spatulata* (fig. 1, *5*), without connecting it to Penard's species. Jankowski (1981) established the genus *Epidiscophrya* with *D. scyphostyla* as the type species and included *D. spatulata* as another representative of the genus. Matthes et al. (1988) considered *D. scyphostyla* as a junior subjective synonym of *D. elongata*. In the present paper, we also assign *D. spatulata* Rieder, 1936 syn. n. to be a junior synonym of *D. elongata*.

Discophrya elongata, mentioned under different species names, was subsequently observed on a variety of different hosts and substrates including aquatic plants (*Riccia* sp.), inanimate substrates (glass slides), and aquatic insects such as the coleopteran *Hydrophilus aterrimus* (Eschscholtz, 1822) and the hemipteran water scorpion *Ranatra linearis* (Linnaeus, 1758) (Dovgal, 1988). Lopez-Ochoterena, Rouré-Cané (1970) and Aladro-Lubel et al. (2007) reported *D. elongata* in Xochimilco Lake and from ponds in Ciudad Universitaria in Mexico City, but no substrates were mentioned. *D. elongata* has been reported as an epibiont of several species of aquatic true bugs (Hemiptera), including *Corisella edulis* (Champion, 1901) and *Notonecta unifasciata* Guérin, 1857 (Marico-Pérez, Mayén-Estrada, 2009; Marico-Pérez et al., 2011). The aim of the present study is to report morphometric variation of *D. elongata* as an epibiont of two species of aquatic true bugs from the same locality in Mexico.

Material and methods

Samples of aquatic true bugs (corixids and notonectids) were collected with a 0.5 mm-mesh dip net from a freshwater pond at Tecomatlán, Hidalgo state, Mexico (20°10'60"N / 99°04'00"W) at 2161 m a. s. l., on several occasions from February to August, 2005. The insects were transported to the laboratory, and some were fixed immediately in 70% ethanol for later identification while others were maintained for several days in aquaria at room temperature. Each basibiont was dissected using a stereoscopic microscope to examine it for the presence of ciliates and allow them to be observed. For light microscopy, body parts were fixed in 70% alcohol and some were stained with Harris's hematoxylin.

Morphometric data of suctorians were recorded, and measurements were checked against Gaussian distribution using the Kolmogorov-Smirnov (K-S), Liliefors, and Shapiro-Wilk's W goodness of fit tests. The normal distribution of the data was confirmed with a high degree of reliability ($p < 0.01$ from the K–S and Liliefors tests and $W = 0.82–0.96$ from the Shapiro-Wilk's test). The maximum, minimum, mean, and standard deviation were calculated. A discriminant analysis and Student's t-test were performed by means of PAST 1.92 software (Hummer et al., 2001) to test variation in the suctorians between the two hosts.

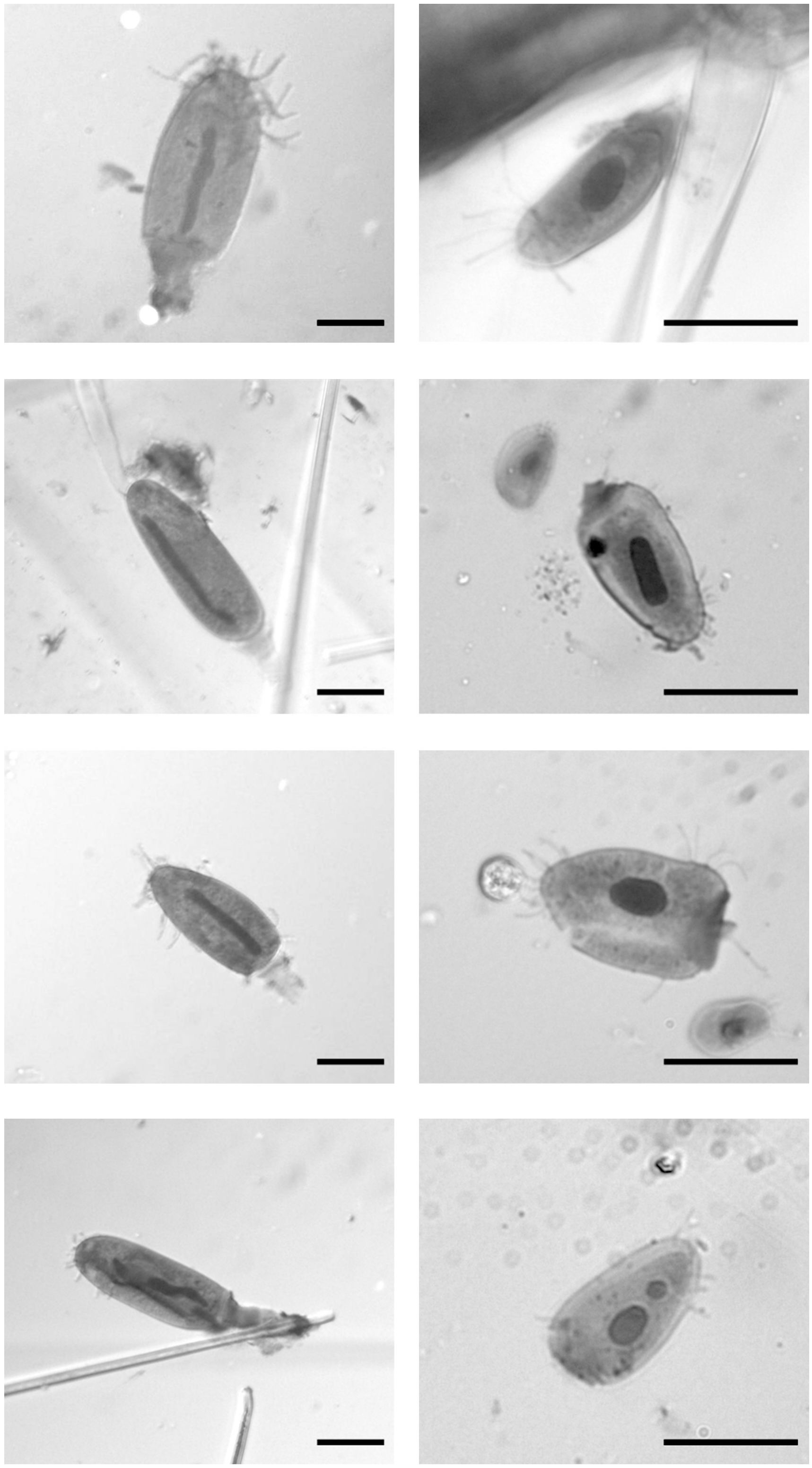


Fig. 2. Individuals of *Discophrya elongata* attached to *Corisella edulis* (right ones) and to *Notonecta unifasciata* (left ones). Scale bars 20 μm.

Рис. 2. Особи *Discophrya elongata* с *Corisella edulis* (правая колонка) и *Notonecta unifasciata* (левая колонка). Масштабные линейки 20 мкм.

Results and discussion

Discophrys elongata was found attached to the legs of *Corisella edulis* (Champion, 1901) (Corixidae) and mainly to the legs of *Notonecta unifasciata* Guérin-Méneville, 1857 (Notonectidae) although in a few cases individuals also were found on the ventral part of its head and abdomen. Individuals of the ciliates attached to *N. unifasciata* were approximately twice as long as those attached to *C. edulis* (table 1 and fig. 2), a trend that was confirmed by a t-test (t-value = −22.05 and p = 0.001). The coefficient of the relationship between length and width was 2.24 ± 0.41 for those on *C. edulis* and 2.72 ± 0.38 for those on *N. unifasciata*, indicating larger size for the suctorians attached to *N. unifasciata*.

The macronuclei of individuals of *D. elongata* attached to *N. unifasciata* were five times as long as those of individuals attached to *C. edulis* (table 1). Differences in the macronucleus width were less evident but still significant. Macronucleus length : width ratio is 1.28 ± 0.28 (on *C. edulis*) and 8.63 ± 2.41 (on *N. unifasciata*); i. e., the suctorians attached to *C. edulis* have spherical macronuclei, whereas the ones attached to *N. unifasciata* have elongate macronuclei.

The symbionts attached to different hosts also differ by the ratio of macronuclear length to length of the entire cell body, which is 0.26 in suctorians attached to *C. edulis* and 0.64 in those attached to *N. unifasciata*. The opposite relationship was seen in the ratio of macronucleus width to width of the entire cell body, 0.446 in ciliates on *C. edulis* and 0.202 in those on *N. unifasciata*.

Calculating the volume of each individual cell was difficult, and we decided to estimate the proportion of the entire macronucleus to the entire organism from the areas (length x width) of each. We found this proportion to be almost invariable among cells within the samples from each host despite the different size of the suctorians: the macronuclear area of *D. elongata* attached to *C. edulis* was 0.119 of the total area versus 0.129 in the suctorians attached to *N. unifasciata*.

Discriminant analysis is resulted in the two canonical variables as shown in fig. 3. Individuals of *D. elongata* from the two host species can be distinguished at a 100% level of confidence based on values of the second canonical variable (Root 2), which can be interpreted as linear dimensions of the cell body and proportions of the macronucleus (table 2). Thus, dimensions of *D. elongata* such as macronuclear length (correlation with

Table 1. Comparision of attributes (mean ± standard deviation) of *Discophrya elongata* on two species of aquatic true bugs from Tecomatlбn, Hidalgo, Mexico (n = 100)

Таблица 1. Сравнение размерных характеристик (среднее ± стандартное отклонение) *Discophrya elongata* с двух видов клопов из пруда возле Текоматлан, Идальго, Мексика (n = 100)

Measurements, μm	*Corisella edulis*	*Notonecta unifasciata*
Total length of cell	29.9 ± 5.9	64.4 ± 14.4
Total width of cell	13.6 ± 3.2	23.8 ± 5.2
Length of macronucleus	7.7 ± 2.2	41.4 ± 13.1
Width of macronucleus	6.1 ± 1.3	4.8 ± 2.4

Table 2. Correlation matrix between dimensions of the body of *Discophrya elongata* and values of two canonical variables (all correlations are significant at p < 0.05)

Таблица 2. Корреляция между размерными характеристиками *Discophrya elongata* и каноническими осями (все коэффициенты корреляции достоверны при p < 0,05)

Dimensions	Root1	Root2
Width of macronucleus	-0.27	-0.56
Length of macronucleus	0.76	0.94
Length of body	0.76	0.89
Width of body	0.71	0.80
Length/width of body	0.58	0.50
Length/width of macronucleus	0.78	0.97

Root 2 values is 0.94), body length (correlation with Root 2 is 0.89), body width (correlation with Root 2 is 0.80), and especially, the ratio of macronucleus length to width (correlation is 0.97) allow individuals from the two hemipteran hosts to be a reliably discriminated from one another.

It is important to note that dimensions of the macronucleus in suctorians are correlated with the cell size, as indicated in the literature (Dovgal, 2002) and exemplified by the population of *D. elongata* surveyed in the present study. Thus, differences in size of the cell body are essentially the result of adaptation of suctorians to colonization of different hosts, whereas variation in size and shape of the macronucleus could result from correlation of these parameters with differences in size of the cell body. Morphometric variation is a common feature among species of ciliates, but this case is interesting because it is a function exclusively of the basibiont they inhabit. A significant statistical difference was obtained in the four characteristics examined.

Dovgal (2000; 2001) concluded that combinations of biotic (host) and abiotic (temperature and salinity) factors influenced in the variability of two species of chonotrich ciliates. In both cases, the host species was an important factor in combination with the abiotic factors mentioned above. These studies were conducted in several localities throughout Ukraine. In the present study, suctorians were collected in the same locality and the same pond (apart from some individuals of *N. unifasciata*), and in all cases, we found that variation of *D. elongata* was strictly correlated with the host species. In this particular case, the host and not abiotic factors (e. g., temperature, pH, aeration) appeared to determine the variation seen in the suctorians.

Some suctorian species such as *Ophryodendron mysidacii* Fernandez-Leborans, Tato-Porto et Sorbe, 1996 have a life cycle with two type of adults (Fernandez-Leborans et al, 1996), but size and shape variations of ciliate species was related with several factors. Starvation followed by intraspecific predation induced cells within a clone to transform into two size classes in *Onychodromus quadricornutus* Foissner, Schlegel et Prescott, 1987 (Wicklow, 1988). For *Lagenophrys aselli* Plate, 1889, Clamp (1988) found two different

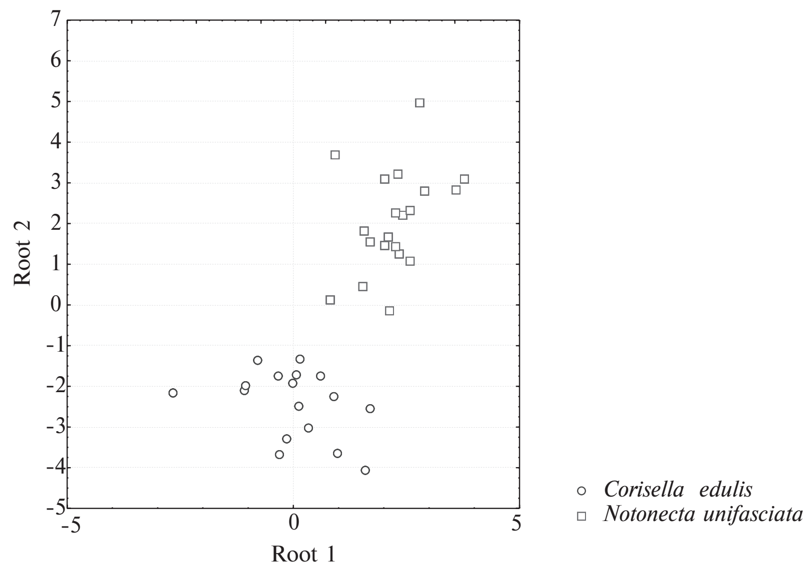


Fig. 3. Scatterplot of canonical scores computed for dimensions of individuals of *Discophrya elongata* from *Corisella edulis* and *Notonecta unifasciata*.

Рис. 3. Распределение особей *Discophrya elongata* с *Corisella edulis* и *Notonecta unifasciata* в пространстве двух первых канонических переменных.

types of individuals, whose morphological variation was attributed to exposure of individuals attaching to different surfaces of the host's pleopods to different flux forces.

In our case, on both host species, individuals *D. elongata* are subjected to hydrodynamic forces. In the case of the more mobile *C. edulis*, these forces are created by the constant movement of the host's legs and possibly could determine the smaller size of its *D. elongata*. By contrast, *N. unifasciata* clings to vegetation and waits for its prey, a habit that may allow the suctorian to grow larger due to less movement of the legs. We also think that taller cells of *D. elongata* in *N. unifasciata* place the tentacles farther out into the flow from the host, and give them a better chance of catching prey from the slower (almost still) flow of water.

The hydrodynamic forces those affect size and shape of commensal suctorians are complicated to calculate, but we think that this pressure is quite significant for the suctorians, as was pointed out in Dovgal's (2008) work, in which the different orientations of *D. elongata* on upper and lateral surfaces of the legs of *Ranatra linearis* were related to the impact of these forces.

One plausible explanation for our observations is, of course, phenotypic plasticity in the suctorian species (Stearns, 1992). In this particular case, the host species could be interpreted as the "habitat", which triggers different responses in the suctorians, an issue that must be researched from the reaction norm approach (Roff, 2002). Such plasticity is perfectly characteristic for *D. elongata*, as evidenced especially by the observations of Collin (1911), who investigated variability of the species using cultures obtained from a few individuals.

Several hypothesis about the way ciliate swarmers select certain substrates or areas of the host body over others have been proposed, i.e. algal exudates may exert an influence in substrate selection by vorticellid telotrochs (Langlois, 1975). Also, selection of certain crustacean host body areas by *Lagenophrys eupagurus* Kellicott, 1893 and *L. crutchfieldi* Clamp, 1993, was attributed to differences in current flow (Clamp, 1973, 1993). In our opinion, colonization of the body of different hosts subjects swarmers of suctorians to different factors that influence their development, producing phenotypes closely adapted to conditions on the host. The swarmers of the following generations are mostly attaching to the same host individuals. In such conditions the acquired phenotypic peculiarities of suctorians might persist during several asexual generations in agreement with concept of long-term modifications characteristic for ciliates (Polyansky, 1982), so size difference would be maintained as long as the lineage remains on the same type of host. Also, the smaller size of the suctorians attached to *Corisella edulis* is due to the factor they fed less and by consequence they divide at a smaller size than those attached to *Notonecta unifasciata*.

A short-term visit of RMP to the Institute of Zoology became possible due to the agreement between the Universidad Nacional Autynoma de México (UNAM) and Schmalhausen Institute of Zoology, NAS of Ukraine. CONACYT (Consejo Nacional de Ciencia y Tecnología) provided scholarships for R. Marico-Pérez and R. Macip-Rí os. Postgrado en Ciencias Biolygicas and Secretaría de Intercambio Académico of the Facultad de Ciencias, UNAM supported I.V. Dovgal for a short-term visit to UNAM. We also wish to thank Paul Tinerella for his help in the identification of nepomorphans.

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